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| <p align="center"><b>8 AMPHETAMINE QUANTITATION AND<br/>CONFIRMATION BY GCMS</b></p>  | <p align="center">Page 1 of 4</p>    |
| <p align="center"><b>Division of Forensic Science</b></p> <p align="center"><b>TOXICOLOGY TECHNICAL PROCEDURES MANUAL</b></p>   | <p>Amendment Designator:</p>         |
|   | <p>Effective Date: 31-March-2004</p> |
| <p align="center"><b>8 AMPHETAMINE QUANTITATION AND CONFIRMATION BY GCMS</b></p> <p><b>8.1 Summary</b></p> <p>Whole blood is made basic with trisodium phosphate buffer and extracted with 1 mL of toluene. The organic layer is then derivatized with trichloroacetylchloride and injected into a GC equipped with an MSD.</p> <p><b>8.2 Specimen Requirements</b></p> <p>8.2.1 2 mL of whole blood, biological fluid or tissue homogenate</p> <p><b>8.3 Reagents And Standards</b></p> <p>8.3.1 NaOH pellets</p> <p>8.3.2 Trisodium phosphate (Na<sub>3</sub>PO<sub>4</sub>)</p> <p>8.3.3 Trichloroacetylchloride</p> <p>8.3.4 Toluene</p> <p>8.3.5 Amphetamine, 1 mg/mL</p> <p>8.3.6 Methamphetamine, 1 mg/mL</p> <p>8.3.7 MDMA, 1 mg/mL</p> <p>8.3.8 MDA, 1 mg/mL</p> <p>8.3.9 p-Chloroamphetamine HCl (internal standard)</p> <p>8.3.10 Methanol</p> <p><b>8.4 Solutions, Internal Standard, Calibrators, Controls</b></p> <p>8.4.1 6.0 N NaOH. Weigh 24 g of NaOH pellets, transfer to a 100 mL volumetric flask and dissolve in dH<sub>2</sub>O. QS to volume with dH<sub>2</sub>O.</p> <p>8.4.2 Saturated trisodium phosphate buffer. Add trisodium phosphate to dH<sub>2</sub>O until no more dissolves after vigorous shaking.</p> <p>8.4.3 dH<sub>2</sub>O/methanol (50:50 v/v) Mix 50 mL dH<sub>2</sub>O with 50 mL methanol.</p> <p>8.4.4 p-Chloroamphetamine HCL stock solution (1 mg/mL): Weigh 12.1 mg p-Chloroamphetamine, transfer to 10 mL volumetric flask and QS to volume with methanol.</p> <p>8.4.5 Working Solution A (10 µg/mL) Add the following to a 10 mL volumetric flask. QS to volume with methanol.</p> <p>8.4.5.1 100 µL of 1 mg/mL amphetamine</p> <p>8.4.5.2 100 µL of 1 mg/mL methamphetamine</p> <p>8.4.5.3 100 µL of 1 mg/mL MDA</p> |                                      |

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| 8.4.5.4  | 100 µL of 1 mg/mL MDMA   |                               |
| 8.4.6  | Working Solution B (1.0 µg/mL)   |                               |
| 8.4.6.1  | Pipet 1.0 mL Working Solution A into a 10 mL volumetric flask. QS to volume with methanol.   |                               |
| 8.4.7  | Working Internal Standard (10µg/mL)  |                               |
| 8.4.7.1  | Pipet 100 µL p-Chloroamphetamine HCL Stock Solution into 10 mL volumetric flask. QS to volume with 50:50 H <sub>2</sub> O/Methanol.  |                               |
| 8.4.8  | Calibrators. The following is an example of an acceptable procedure for the preparation of calibrators. Other quantitative dilutions may be acceptable to achieve similar results. |                               |
| 8.4.8.1  | To 16 x 125 mm screw-cap test tubes, add 1 mL certified negative blood, then add the following amounts of Working Solution and vortex for 15 seconds.                              |                               |
| 8.4.8.1.1  | 2.00 mg/L = 200 µL of Working Solution A   |                               |
| 8.4.8.1.2  | 1.00 mg/L = 100 µL of Working Solution A   |                               |
| 8.4.8.1.3  | 0.50 mg/L = 50 µL of Working Solution A  |                               |
| 8.4.8.1.4  | 0.10 mg/L = 100 µL of Working Solution B   |                               |
| 8.4.8.1.5  | 0.05 mg/L = 50 µL of Working Solution B  |                               |
| 8.4.8.1.6  | 0.02 mg/L = 20 µL of Working Solution B  |                               |
| 8.4.8.1.7  | 0.01 mg/L = 10 µL of Working Solution B  |                               |
| 8.4.9  | Controls   |                               |
| 8.4.9.1  | Negative control blood: blood bank blood or equivalent determined not to contain amphetamines  |                               |
| 8.4.9.2  | In house amphetamine control is prepared from a different lot number or different manufacturer of amphetamines.  |                               |
| 8.4.9.3  | Commercial whole blood control (QAS or other commercial vendor).   |                               |
| 8.5  | <b>Apparatus</b>   |                               |
| 8.5.1  | Gas chromatograph with mass selective detector   |                               |
| 8.5.2  | Test tubes, 16 x 125 mm round bottom, screw cap tubes, borosilicate glass with Teflon caps   |                               |
| 8.5.3  | Test tubes, 13 x 100 mm round bottom tubes, borosilicate glass   |                               |
| 8.5.4  | Centrifuge capable of 2,000 – 3,000 rpm  |                               |
| 8.5.5  | Vortex mixer   |                               |
| 8.5.6  | Test tube rotator  |                               |

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| 8.5.7  | GC autosampler vials and inserts  |   |
| 8.5.8  | HP GC/MSD   |   |
| 8.5.8.1  | Acquisition Mode: SIM   |   |
| 8.5.8.2  | Amphetamine TCA:  | <u>118</u> , 188, 190   |
| 8.5.8.3  | Methamphetamine TCA:  | <u>204</u> , 202, 91, 118   |
| 8.5.8.4  | MDA TCA:  | <u>162</u> , 135, 190   |
| 8.5.8.5  | MDMA TCA:   | <u>162</u> , 202, 204   |
| 8.5.8.6  | p-Chloroamphetamine TCA:  | <u>190</u>  |
| 8.5.8.7  | Column:   | Phenomenex 15MX 15 m x 0.25 mm x 0.25 µm  |
| 8.5.8.8  | Detector Temperature:   | 280 °C  |
| 8.5.8.9  | Instrument conditions may be changed to permit improved performance.  |   |
|  | 8.5.8.9.1   | Oven Program  |
|  |   | <ul style="list-style-type: none"> <li>• Equilibration time: 0.50 minutes</li> <li>• Initial temp: 110 °C</li> <li>• Initial time: 0.5 minutes</li> <li>• Ramp 20 °C/min</li> <li>• Final Temp 280 °C</li> <li>• Final Time 8 minutes</li> <li>• Run Time 17 minutes</li> </ul> |
|  | 8.5.8.9.2   | Inlet   |
|  |   | <ul style="list-style-type: none"> <li>• Mode: Splitless</li> <li>• Temperature: 260 °C</li> <li>• Injection volume: 2 µL</li> <li>• Purge Time ON at 2 minutes</li> </ul>  |
| <b>8.6</b>   | <b>Procedure</b>  |   |
| 8.6.1  | Label clean 16 x 125 mm screw cap tubes accordingly, negative, calibrators, control(s) and case sample IDs.                           |   |
| 8.6.2  | Pipet 1 mL of blank blood, calibrators, controls and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes. |   |
| 8.6.3  | Add 100 µL internal standard into all tubes and vortex briefly.   |   |
| 8.6.4  | Add 2 mL saturated trisodium phosphate buffer to each tube. Vortex briefly.   |   |
| 8.6.5  | Add 1 mL toluene to each tube.  |   |
| 8.6.6  | Rotate for 30 minutes at slow speed.  |   |

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| <p>8.6.7 Centrifuge at 2800 rpm for 15 minutes.</p> <p>8.6.8 Transfer upper layer (toluene) to clean, 13 x 100 mm screw-cap tube. Add 1 drop of trichloroacetylchloride to each tube, cap, and vortex gently.</p> <p>8.6.9 Let samples sit for 1 hour at room temperature.</p> <p>8.6.10 Add 2 mL of 6N NaOH to each tube.</p> <p>8.6.11 Vortex vigorously for 30 seconds.</p> <p>8.6.12 Centrifuge for 10 minutes at 2800 rpm.</p> <p>8.6.13 Transfer an aliquot of upper (toluene) layer into GC autosampler vial.</p> <p>8.6.14 Inject 2 µL onto GCMS.</p> <p><b>8.7 Calculation</b></p> <p>8.7.1 Calculate the concentrations by interpolation of a linear plot of the response curve based on peak height (or area) ratios (using the target ions listed under GCMS conditions) versus calibrator concentration.</p> <p>8.7.2 Qualifier ion ratio range. The qualifier ion ratio range is calculated by determining the mean ± 20% (or 2 SD) ion ratio from all calibrators used in the calibrations curve.</p> <p><b>8.8 Quality Control And Reporting</b></p> <p>8.8.1 See Toxicology Quality Guidelines</p> <p><b>8.9 References</b></p> <p>8.9.1 "Amphetamine and Methamphetamine" by Wayne Harrington, <u>Methodology of Analytical Toxicology</u> by Irving Sunshine(Ed), CRC Press, 1975.</p> |                                      |